MICROSTIMULATION OF LUMBOSACRAL SPINAL CORD-MAPPING

Contract #N01-NS-8-2301

2nd Progress Report
January 1, 1999 to March 31, 1999
Neural Prosthesis Program

Prepared for
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I. Introduction

New experiments initiated during the previous quarter continued during this quarter. These experiments examined sites which could modulate colon activity by microstimulation of specific areas in the sacral spinal cord using fine tipped microelectrodes. The sites in the spinal cord which became targets for microstimulation were, in part, identified by tracing experiments using pseudorabies virus (PRV). Two abstracts summarizing the colon microstimulation and PRV tracing experiments have been submitted for presentation at the 1999 Society for Neuroscience meetings. Copies of these abstracts are attached to this progress report. The data presented in these abstracts show that microstimulation in the S_2 and S_3 spinal cord in and near the sacral parasympathetic nucleus could produce changes in colon intraluminal pressure, especially in the distal colon. These changes were often small however, when compared to ventral root simulation. In addition the stimulus threshold for activation of the colon was typically higher than those seen in previous studies of penile erection and bladder modulation.

The colon tracing studies indicate that sites which contain colon preganglionic neurons or their axons produced the largest amplitude responses, while sites which contained primarily interneurons (near the central canal and in the dorsal commissure) produced either no response or very small responses.

During this quarter the construction of an 8-channel stimulator was also begun. This stimulator is computer controlled and will allow the activation of multiple spinal cord sites using several (2-8) microelectrodes. This stimulator will have a variety of advantages when examining the effects of multiple electrode stimulation at various stimulus parameters.

II. Modulation of Colon Intraluminal Pressure by Sacral Spinal Cord Microstimulation

These studies were designed to examine sites in the sacral spinal cord which produce colon contractions to focal microstimulation and to examine the stimulus parameters which produce large amplitude responses with minimal fatigue and non-specific responses.

The initial methods used in our early experiments have been somewhat modified. The major changes in our most recent experiments are summarized below.

In our initial experiments, intraluminal colon pressure changes were recorded form a single

saline filled balloon attached to the tip of a double lumen catheter and placed in the distal colon approximately 6cm from the end of the anal canal. This position of the balloon catheter was chosen since it was known, from studies in this and other laboratories, that the sacral spinal cord provides the major innervation to mid and distal colon and to a smaller extent, the proximal colon. The balloon was also positioned to avoid the last few centimeters of the colon (anal canal), since this area of the distal colon contains functionally specialized smooth and striated muscles of the internal and external anal sphincter. This segment of the large intestine is important to the storage and elimination functions of the gastrointestinal tract and will be examined in another series of experiments.

In our most recent experiments two balloon catheters were used to record intraluminal pressure. One positioned in the distal colon and the other in the proximal colon (see Fig. 1). Although the pressure changes in the proximal colon have been small, it was thought to be important to determine the extent to which the proximal colon could be activated by spinal cord microstimulation, either directly or reflexly.

The changes in colon intraluminal pressure to sacral spinal cord microstimulation using a single microelectrode have been small in the range of 5 - 15 cm H_2O. Even with ventral root stimulation the intraluminal pressure changes, although often large (20 - 40 cm H_2O), have been quite variable among animals. The spontaneous colonic activity has been small and quite variable as well. We, therefore, explored a variety of manipulations to enhance the stimulus activated colon contractions. We were not interested in increasing spontaneous contractions, since large, long duration spontaneous contractions could interfere with mapping of sites in the spinal cord. The various manipulations included the use of various types and combinations of anesthetics and the sectioning of certain sympathetic nerves which are inhibitory to colonic activity. These are methods suggested and commonly used in published literature to reestablish gastrointestinal motility which has been suppressed by anesthesia or surgical manipulation of the G.I. tract.

We have used both pentobarbital (25 - 35mg/kg iv) and ∝-chloralose (60 - 70 mg/kg iv) in our experiments. Although both anesthetics have been used extensively for colon experiments, neither anesthetic seemed to have a dramatic advantage when recording stimulus, evoked or spontaneous, colonic activity.

It is known that activation of the sympathetic input to colon can suppress colon contractions. In these animals we therefore cut the hypogastric and lumbar colonic nerves bilaterally, which supply the proximal and distal colon. This manipulation had little effect on either stimulus evoked response or spontaneous contractions, although a slight increase in spontaneous activity was seen in one animal

Although two different anesthetics were used in our experiments and sympathetic were either cut or remained intact, there seemed to be little difference in the colonic contractions recorded. Therefore the results form these various preparations will be combined when drawing conclusions from these experiments. The experimental preparation which will be used in future experiments will use pentobarbital anesthesia with intact innervation.

III. Results

In order to determine the spinal cord segments to examine with focal microstimulation each sacral ventral root is stimulated to identify the root which produces the largest response. The assumption is that the ventral root which produces the largest amplitude response to varying intensities of stimulation is attached to the spinal segment which provides the major excitatory input to the colon. Figure 2 shows the response for a typical experiment in which the sacral (S_1, S_2, and S_3) ventral roots were stimulated at various intensities. Stimulation of S_1 ventral root produced no response and is not shown. In this particular experiment the S_3 ventral root gave a slightly larger response than S_2. The proximal colon produced no response to ventral root stimulation and are not plotted on the graph. The peak amplitude, as well as the area under the colon contraction curve and duration of the response to a 30 second stimulus (15Hz, 0.05 msec pulse duration) are plotted on each graph. The plot at the bottom of Figure 2 is a repeat of S_3 ventral root simulation using a pulse duration of 0.2 msec.

In this particular experiment the S_3 spinal cord segment was mapped first with fine tipped microelectrodes since it had a slightly larger response to ventral root stimulation than S_2. Figure 3 shows records from four electrode tracts (1,2, 5, & 6) in the S_3 spinal cord from the same animal as Figure 2. In this figure the specific colon responses are seen at about 1.0 mm from the cord surface and continues to a depth of about 2.2 mm. Tracts 1 and 2 (Figure 3) with the electrode trajectory

passing through, or slightly medial to the sacral parasympathetic nucleus (SPN) produce the most specific colon response. The responses seen at the bottom of tract 5 and 6, deep in the ventral horn, are colon responses accompanied by somatic leg movement and histologically lie near or outside the edge of the cord. Very lateral tracts (3 and 4, Figure 3, with response not shown) produce no specific colon contraction although in some animals small contractions can be seen deep as the electrode tip passes through the ventral surface of the spinal cord. The responses seen very superficially at the surface of the cord (Figure 3, tract 2) probably represent reflex activation of the colon due to afferent simulation.

The intensity threshold for stimulation both in the spinal cord and on the ventral roots, was higher for colon responses than for other autonomic functions such as bladder contraction or penile erection. This is likely due to the high threshold small unmyelinated C-fiber innervation of the colon, while bladder and penile are innervated by myelinated fibers.

These mapping experiments will continue into the next quarter, with experiments designed to examine the external anal sphincter and lower anal canal.

IV. Development of Eight Channel Stimulator

Although stimulation of a single site in the spinal cord can often produce a small end organ response, a robust functional response seems to require several electrode sites activated simultaneously or in some special pattern. During this quarter we've begun development of a multichannel stimulator having a large amount of flexibility to change patterns and parameters of stimulation. The design criteria for the stimulator included the following: (1) a minimum of six channels was felt were needed (final design had eight); (2) the ability to change any stimulus parameters on all or any single electrode with a short delay of 25 msec or less. The ideal would be to change at least one parameter before the next stimulus pulse occurs for a 50 or 100Hz frequency of stimulation; (3) the ability to feedback information from the response to modify the stimulus parameters; (4) to be able to deliver a complex pattern of stimulation to single or several electrodes and to repeat a given pattern for a specified time period; and (5) to be able to replace a standard laboratory stimulator.

A stimulator that seems to meet most of the above criteria has now been implemented on a

Pentium 333 MHz PC using an 8-channel high speed D/A card. We have tested this stimulator in one animal experiment switching between it and a standard Grass S88 stimulator throughout the experiment. The responses appear identical for the two stimulators for any given stimulus over a wide range of parameters.

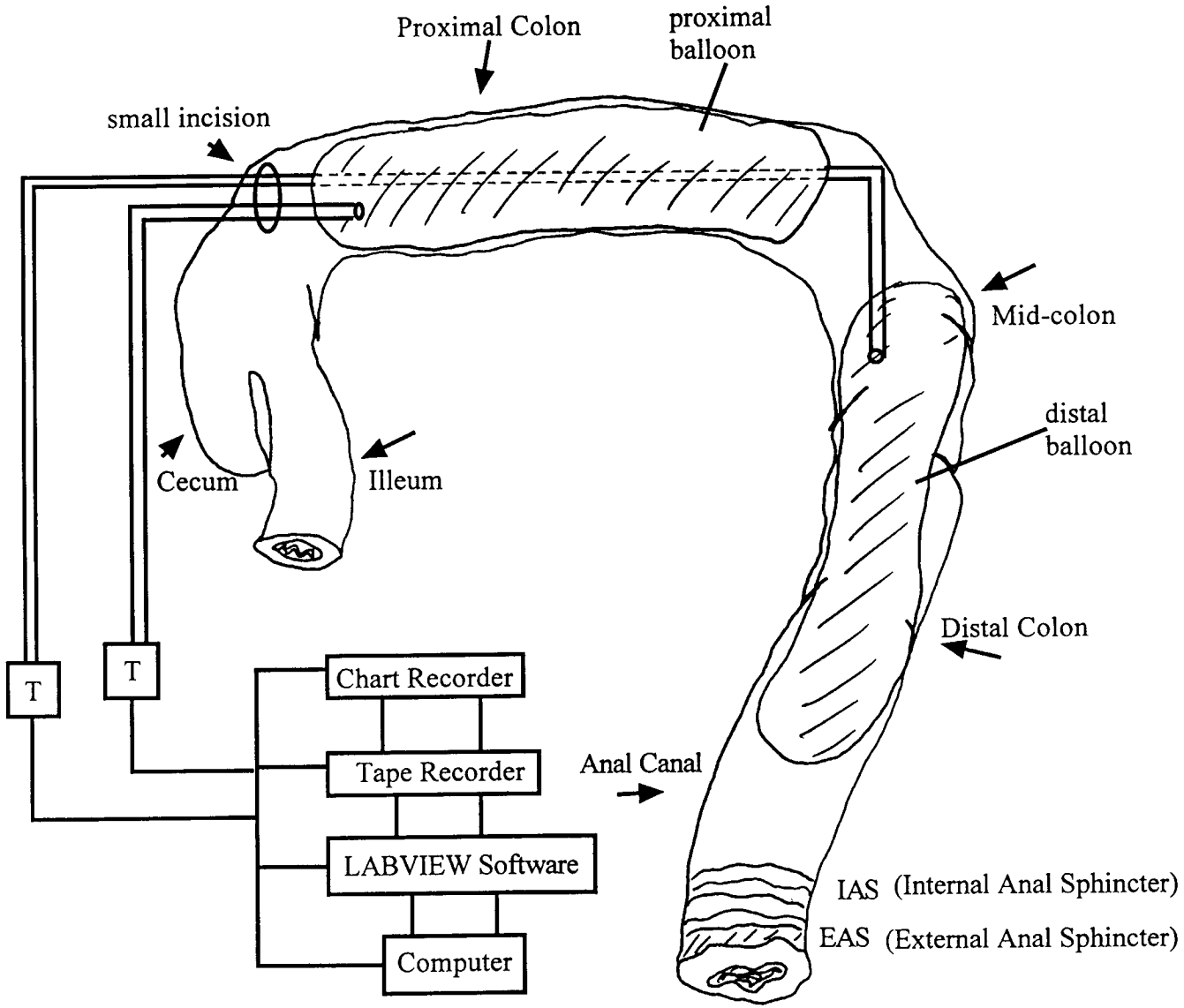
By installing a data acquisition card in the same PC, response information can be used to modify the stimulus parameters and simultaneously save the responses and stimulus information in memory or on disk. The exact limits of the system in terms of data acquisition rate and rate of updating stimulus parameters, are not exactly known at the present time. However, it appears that useful rates such as sampling torque at 100 samples/sec, updating the stimulus intensity on four electrodes every 100 msec, and simultaneously saving all the data to disk, is a relatively easy task.

The further development of this stimulator and its use in additional animal experiments will continue during the next quarter.

Figure 1 Schematic diagram of the methods used for recording colon intraluminal pressure changes. Two saline filled balloons are inserted into the colon via a small incision in the proximal colon. One balloon is positioned in the middle and distal colon about 6 cms from the anal opening so as to avoid the internal and external anal sphincters. The other balloon is positioned in the proximal colon just caudal to junction of the ileum. The saline filled balloon catheters are connected to transducers and their signals recorded on paper (chart recorder), on tape, and digitized and stored on the computer disk drive. The computer provides various displays during the experiment and also is used for analysis following completion of the experiment.

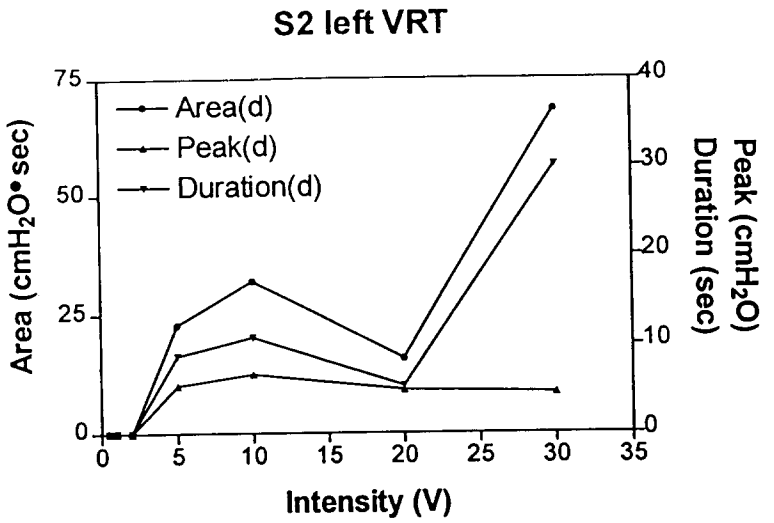
Figure 2 Plots of colon responses to stimulation of the S_2 (top) and S_3 (middle & bottom) ventral roots to increasing intensities of stimulation. Only responses of distal (d) colon are shown since proximal colon produced no response. Responses include peak distal colon contraction (Peak (d)), area under the colon contraction curve (Area (d)), and duration of colon contraction (Duration (d)). Stimulus parameters for S_2 (top) and S_3 (middle) are: 15Hz, 0.05 msec pulse duration, 0.1 - 30 V, 30 sec on 120 sec off. For S_3 (bottom) same except 0.2 msec pulse duration. S_1 gave no response and is not shown.

Figure 3 Plots of colon responses to focal microstimulation at various depths form the surface of the S_3 spinal cord. Responses along four electrode tracts (1, 2, 5, & 6) are shown at 200μ increments. The trajectory of the tracts in S_3 spinal cord are illustrated by the spinal cord figurine at the center of the figure. Response along tracts 2 & 4 are not shown (see text). Responses are same as Figure 2. Area, peak, and duration each for proximal (p) colon recording site and distal (d) site. Stimulus parameters are: 100μA, 0.2 msec pulse duration, 15Hz, 30 sec on 120 sec off. Responses at 2.8 mm or below are near or outside the edge of the spinal cord and represent non-specific activation of colon as well as somatic responses. Specific colon responses are between 1.0 and 2.2 mm in tracts 1, and 2.



T = Transducer

Figure 1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Area(d)
Peak(d)
Duration(d)
Area (cmH2O•sec)
Peak (cmH2O)
Duration (sec)
Intensity (V)
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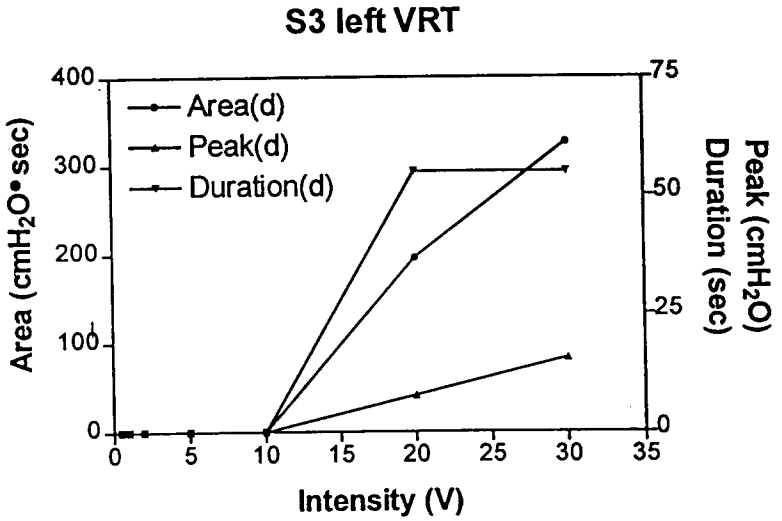
25
50
75
0
10
20
30
40
0
5
10
15
20
25
30
35


S3 left VRT
Area(d)
Peak(d)
Duration(d)
Area (cmH2O•sec)
Peak (cmH2O)
Duration (sec)
Intensity (V)
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100
200
300
400
0
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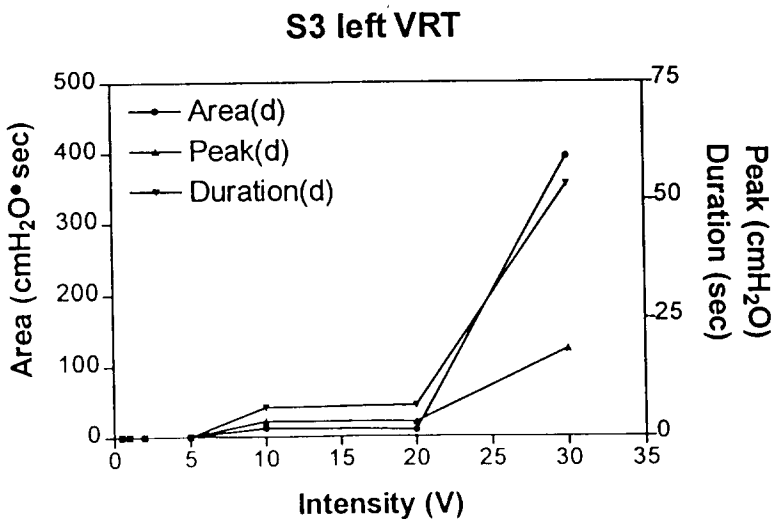
50
75
0
5
10
15
20
25
30
35


S3 left VRT
Area(d)
Peak(d)
Duration(d)
Area (cmH2O•sec)
Peak (cmH2O)
Duration (sec)
Intensity (V)
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100
200
300
400
500
0
25
50
75
0
5
10
15
20
25
30
35

Figure 2

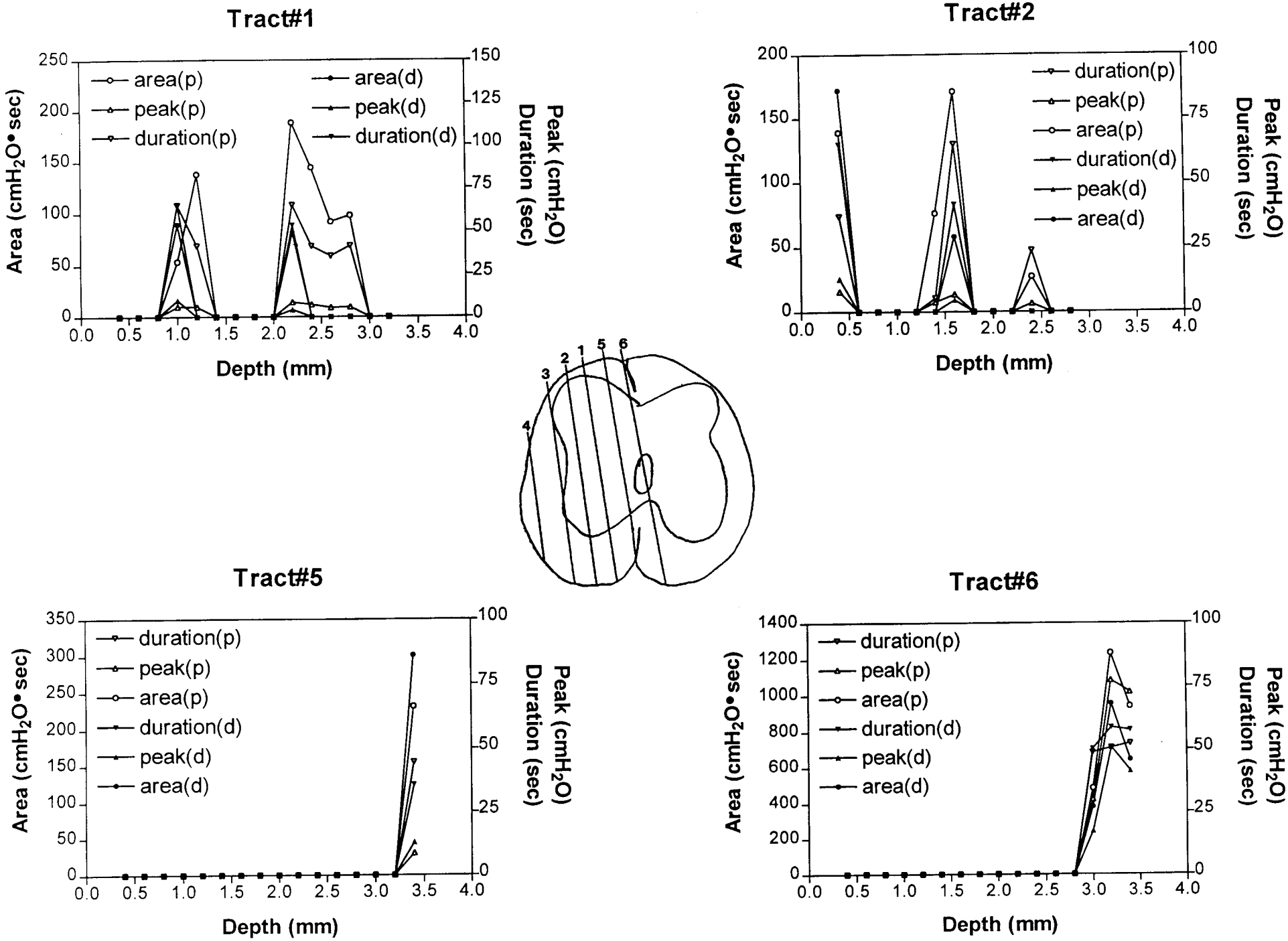
Tract#1
Area (cmH2O•sec)
Peak (cmH2O)
Duration (sec)
area(p)
peak(p)
duration(p)
area(d)
peak(d)
duration(d)
Depth (mm)
Tract#2
Area (cmH2O•sec)
Peak (cmH2O)
Duration (sec)
duration(p)
peak(p)
area(p)
duration(d)
peak(d)
area(d)
Depth (mm)
1
2
3
4
5
6
Tract#5
Area (cmH2O•sec)
Peak (cmH2O)
Duration (sec)
duration(p)
peak(p)
area(p)
duration(d)
peak(d)
area(d)
Depth (mm)
Tract#6
Area (cmH2O•sec)
Peak (cmH2O)
Duration (sec)
duration(p)
peak(p)
area(p)
duration(d)
peak(d)
area(d)
Depth (mm)

Figure 3

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1st topic title: Gastrointestinal & Urogenital Regulation **topic number:** 70

2nd theme title: Motor Systems & Sensorimotor **theme letter:** G
2nd topic title: Spinal Cord & Brainstem **topic number:** 94

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MODULATION OF COLON INTRALUMINAL PRESSURE BY MICROSTIMULATION OF THE SACRAL SPINAL CORD.

C. TAI*, A.M. BOOTH, W.C. de GROAT & J.R. ROPPOLO. DEPT. OF PHARMACOLOGY, UNIVERSITY OF PITTSBURGH, SCHOOL OF MEDICINE, PITTSBURGH, PA 15261

The purpose of this study was to determine the stimulation parameters and the location of sites within the sacral spinal cord where focal electrical stimulation produced colon contractions in the cat. Adult male cats anesthetized with either pentobarbital (35mg/kg) or ∝-chlorolose (60mg/kg) were used in this study. Intraluminal colon pressure (ICP) was recorded via two saline filled balloon catheters, one placed in the distal colon and the other in the proximal colon. Each sacral ventral root (S_1 - S_3) was stimulated with a hook electrode to determine the spinal segment which produced the largest amplitude ICP response. Stimulation of the S_2 and S_3 ventral roots typically produced the largest ICP response ranging from 15 - 40 cm H_2O pressure in distal colon and much smaller (5 - 10 cm H_2O) ICP or no response in proximal colon. The S_1 ventral root produced very small or no ICP responses. The segments (S_2 and S_3) which produced the largest ICP response were probed with fine-tipped (300 - 400μ^2 surface area) activated iridium microelectrodes advanced from the dorsal surface of the spinal cord in 200μ increments. Stimulation sites which produced the largest ICP responses were 1.2 - 2.2 mm from the S_2 or S_3 cord surface and 300 - 400μ medial to the lateral edge of the grey matter. Superficial sites near the cord surface at the dorsal root entry zone also produced small responses which were thought to be reflexes due to afferent stimulation. The responses evoked by spinal cord stimulation occurred at a latency of 3 - 15 seconds and had an amplitude of 10 - 20 cm H_2O. The frequency and intensity of microstimulation were also important in producing maximal response. The optimal frequency was between 10 and 20 Hz using 0.2 msec pulses at an intensity of 50 - 150μA. The threshold for colon activation was 50 - 60μA, typically higher than those for bladder and penile responses seen in our previous experiments. These studies suggest that colon responses are more difficult to elicit by cord microstimulation than either bladder or penile responses, and may require several electrodes positioned in the sacral cord to produce a larger response.

Supported by NIH/NINDS contract # N01-NS-8-2301.

Key Words: (see instructions p. 4)

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TRANSNEURONAL LABELING OF NEURONS IN THE CAT SPINAL CORD FOLLOWING INJECTION OF PSEUDORABIES VIRUS INTO THE COLON. J.R. ROPPOLO*, V. L. ERICKSON, C. TAI, A.M. BOOTH, P. CARD & W.C. de GROAT. DEPT. OF PHARMACOLOGY, UNIVERSITY OF PITTSBURGH, SCHOOL OF MEDICINE, PITTSBURGH, PA 15261

The purpose of this study was to determine the location and distribution of spinal efferent neurons and interneurons which control colonic function. Pseudorabies virus (PRV, Becker strain 7 x 10^8ppu/ml) was injected into the distal and middle colon of halothane anesthetized male cats. In order to avoid injections into the striated muscle of the external anal sphincter the distal most 6cm (measured from anus) was not injected. Fifty-5μl injections of PRV were made into the remaining distal and middle colon. Animals were perfused with fixative 80-104 hrs following injection and the spinal cord tissue cut, reacted with PRV antibody and processed using standard techniques. PRV infected (PRV-I) neurons were located at several levels of the spinal cord and dorsal root ganglion (DRG) including: sacral (S_1 - S_3), lumbar (L_1 - L_4) and thoracic (T_{13}) levels. The majority of PRV labeled neurons were seen in sacral(S_2 & S_3) & lumbar (L_2, L_3, & L_4) segments of spinal cord and DRG. In the sacral cord, PRV-I neurons were seen medial and dorsal in sacral parasympathetic nucleus (SPN) although a few PRV-I neurons were also seen near the lateral edge, primarily in the S_3 segment. PRV-I interneurons were located near the central canal (CC) and in the dorsal commissure (DC). The SPN of the caudal S_1 segment contained only a few PRV-I neurons, however labeling in DC and around the CC extended to middle and rostral S_1. No labeled neurons were observed in Onuf's nucleus or deep in the ventral horns. PRV-I neurons were seen in lumbar spinal cord in the intermediolateral cell column (IML) near the lateral edge of the grey matter. PRV-I labeled neurons are also found near the CC and DC. In many sections the area between the IML and CC contained a few neurons forming a bridge between the IML and CC. The location of labeled PRV-I efferent neurons and interneurons from the colon is similar to that seen with PRV labeling in the urogenital system.

Supported NIH/NINDS contract #N01-NS-8-2301.

Key Words: (see instructions p. 4)

1. dorsal root ganglia (DRG)
2. colon efferents
3. interneurons
4. mapping

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